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<b>(21) International Application Number:</b> PCT/CA96/00638 <b>(22) International Filing Date:</b> 24 September 1996 (24.09.96)  <b>(30) Priority Data:</b> 60/004,418 27 September 1995 (27.09.95) US 9605152.9 12 March 1996 (12.03.96) GB  <b>(71) Applicant (for all designated States except US):</b> MERCK-FROSST CANADA INC. [CA/CA]; 16711 Trans Canada Highway, Kirkland, Quebec H9H 3L1 (CA). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> PRASIT, Petpiboon [CA/CA]; 16711 Trans Canada Highway, Kirkland, Quebec H9H 3L1 (CA). YOUNG, Robert, N. [CA/CA]; 16711 Trans Canada Highway, Kirkland, Quebec H9H 3L1 (CA).  <b>(74) Agent:</b> MURPHY, Kevin, P.; Swabey Ogilvy Renault, Suite 1600, 1981 McGill College, Montreal, Quebec H3A 2Y3 (CA).		<b>(81) Designated States:</b> AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> COMPOSITIONS FOR TREATING INFLAMMATION CONTAINING CERTAIN PROSTAGLANDINS AND A SELECTIVE CYCLOOXYGENASE-2 INHIBITOR		
<b>(57) Abstract</b>  A method of treating cyclooxygenase mediated disease while promoting the healing of certain lesions including gastric ulcers comprising the co-administration of certain prostaglandins and a selective cyclooxygenase-2 inhibitor, or the co-administration of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor as defined herein.		

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COMPOSITIONS FOR TREATING INFLAMMATION CONTAINING CERTAIN PROSTAGLANDINS AND A  
SELECTIVE CYCLOOXYGENASE-2 INHIBITOR

BACKGROUND OF THE INVENTION

5                   This invention relates to a method of treating  
cyclooxygenase mediated diseases in patients with ulcers. Disclosed is a  
method of treating cyclooxygenase mediated disease while promoting  
the healing of certain lesions including gastric ulcers comprising the co-  
administration of certain prostaglandins and a selective cyclooxygenase-  
10   2 inhibitor, or the co-administration of an anti-ulcer agent and a  
selective cyclooxygenase-2 inhibitor as defined below.

Non-steroidal, anti inflammatory drugs exert most of their  
anti inflammatory, analgesic and antipyretic activity and inhibit  
hormone-induced uterine contractions and certain types of cancer  
15   growth through inhibition of prostaglandin G/H synthase, also known as  
cyclooxygenase. Up until recently, only one form of cyclooxygenase  
had been characterized, this corresponding to cyclooxygenase-1 or the  
constitutive enzyme, as originally identified in bovine seminal vesicles.  
Recently the gene for a second inducible form of cyclooxygenase  
20   (cyclooxygenase-2) has been cloned, sequenced and characterized from  
chicken, murine and human sources. This enzyme is distinct from the  
cyclooxygenase-1 which has now also been cloned, sequenced and  
characterized from sheep, murine and human sources. The second form  
of cyclooxygenase, cyclooxygenase-2, is rapidly and readily inducible  
25   by a number of agents including mitogens, endotoxin, hormones,  
cytokines and growth factors. As prostaglandins have both  
physiological and pathological roles, we have concluded that the  
constitutive enzyme, cyclooxygenase-1, is responsible, in large part, for  
endogenous basal release of prostaglandins and hence is important in  
30   their physiological functions such as the maintenance of gastrointestinal  
integrity and renal blood flow. In contrast, we have concluded that the  
inducible form, cyclooxygenase-2, is mainly responsible for the  
pathological effects of prostaglandins where rapid induction of the  
enzyme would occur in response to such agents as inflammatory agents,

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hormones, growth factors, and cytokines. Thus, a selective inhibitor of cyclooxygenase-2 will have similar anti inflammatory, antipyretic and analgesic properties to a conventional non-steroidal anti inflammatory drug, and in addition would inhibit hormone-induced uterine  
5 contractions and have potential anti-cancer effects, but will have a diminished ability to induce some of the mechanism-based side effects. In particular, such a compound should have a reduced potential for gastrointestinal toxicity, a reduced potential for renal side effects, a reduced effect on bleeding times and possibly a lessened ability to induce  
10 asthma attacks in aspirin-sensitive asthmatic subjects.

US 5,015,481, issued May 14, 1991 discloses the use of defined combinations of NSAID's and prostaglandins for the prevention of NSAID induced ulcers. WO 91/16896, published November 14, 1991 discloses defined combinations of NSAID's and prostaglandins to  
15 treat mild to moderate pain. WO 91/16895, published November 14, 1991 discloses a pharmaceutical composition including a core of an NSAID selected from diclofenac and piroxacam which core is surrounded by a mantle coating of a prostaglandin, wherein an intermediate coating can be present between the NSAID core and the  
20 prostaglandin mantle coating. US 5,232,704 discloses a sustained release dosage form of prostaglandin which in combination which is said to be useful for the prevention of NSAID induced ulcers.

More recently, it has been disclosed that NSAID induced gastric ulcers are caused by the cyclooxygenase-1 activity found in most  
25 NSAID's. Accordingly, the treatment of cyclooxygenase-2 mediated diseases by administration of an NSAID that selectively inhibits cyclooxygenase-2 in substantial preference to cyclooxygenase-1 eliminates the advantage of co-administering prostaglandin with the NSAID for purposes of preventing NSAID induced ulcers. Surprisingly,  
30 we have found that in addition to its negative role in the inflammatory process, cyclooxygenase-2 also plays an important positive role in gastric mucosal protection and in promoting the healing of certain lesions including gastric ulcers. Accordingly, the applicants disclose a method of treating cyclooxygenase mediated disease while promoting

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the healing of certain lesions including gastric ulcers comprising the co-administration of certain prostaglandins and a selective cyclooxygenase-2 inhibitor, or the co-administration of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor as defined below.

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### SUMMARY OF THE INVENTION

A method of treating cyclooxygenase mediated disease while promoting the healing of certain lesions including gastric ulcers and protecting the gastric mucosa comprising the co-administration of certain prostaglandin and a selective cyclooxygenase-2 inhibitor, or the co-administration of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor as defined below.

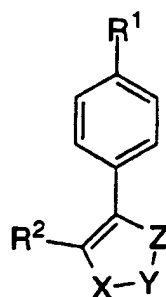
15 DETAILED DESCRIPTION OF THE INVENTION

The invention encompasses a method of treating cyclooxygenase mediated disease while promoting the healing of certain lesions including gastric ulcers comprising the co-administration of a prostaglandins and a selective cyclooxygenase-2 inhibitor, or the co-administration of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor as defined below.

For purposes of this specification a compound shall be defined as a selective cyclooxygenase-2 inhibitor if the ratio of it's IC<sub>50</sub> for the inhibition of cyclooxygenase-1 divided by it's IC<sub>50</sub> for the inhibition of cyclooxygenase-2, as measured as described in this specification or a comparable method is 200 or greater; preferably 1000 or greater.

Accordingly, for purposes of this specification selective cyclooxygenase-2 inhibitors includes, but is not limited to compounds of Formula I

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I

or a pharmaceutically acceptable salt thereof wherein:

5 X-Y-Z is selected from the group consisting of:

- (a) -C(O)-O-CR<sup>5</sup>(R<sup>5'</sup>)-,
- (b) -C(O)-CH<sub>2</sub>-CR<sup>5</sup>(R<sup>5'</sup>),
- (c) -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-

10 R<sup>1</sup> is selected from the group consisting of

- (a) S(O)<sub>2</sub>CH<sub>3</sub>,
- (b) S(O)<sub>2</sub>NH<sub>2</sub>,
- (c) S(O)<sub>2</sub>NHC(O)CF<sub>3</sub>,

R<sup>2</sup> is selected from the group consisting of

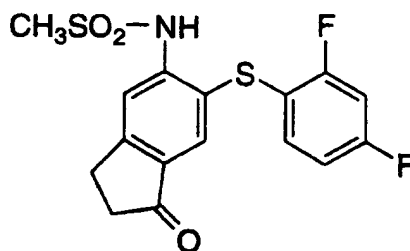
- 15 (a) C<sub>1</sub>-6alkyl,
- (b) C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, and C<sub>7</sub>, cycloalkyl,
- (c) mono-, di- or tri-substituted phenyl or naphthyl wherein the substituent is selected from the group consisting of
  - (1) hydrogen,
  - 20 (2) halo,
  - (3) C<sub>1</sub>-6alkoxy,
  - (4) C<sub>1</sub>-6alkylthio,
  - (5) CN,
  - (6) CF<sub>3</sub>,
  - 25 (7) C<sub>1</sub>-6alkyl,
  - (8) N<sub>3</sub>,
  - (9) -CO<sub>2</sub>H,
  - (10) -CO<sub>2</sub>-C<sub>1</sub>-4alkyl,

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- (11) -C(R<sup>3</sup>)(R<sup>4</sup>)-OH,  
(12) -C(R<sup>3</sup>)(R<sup>4</sup>)-O-C<sub>1-4</sub>alkyl, and  
(13) -C<sub>1-6</sub>alkyl-CO<sub>2</sub>-R<sup>3</sup>;
- (d) mono-, di- or tri-substituted heteroaryl wherein the  
5 heteroaryl is a monocyclic aromatic ring of 5 atoms, said  
ring having one hetero atom which is S, O, or N, and  
optionally 1, 2, or 3 additionally N atoms; or  
the heteroaryl is a monocyclic ring of 6 atoms, said ring  
10 having one hetero atom which is N, and optionally 1, 2, 3,  
or 4 additional N atoms; said substituents are selected from  
the group consisting of  
(1) hydrogen,  
(2) halo, including fluoro, chloro, bromo and iodo,  
(3) C<sub>1-6</sub>alkyl,  
15 (4) C<sub>1-6</sub>alkoxy,  
(5) C<sub>1-6</sub>alkylthio,  
(6) CN,  
(7) CF<sub>3</sub>,  
(8) N<sub>3</sub>,  
20 (9) -C(R<sup>3</sup>)(R<sup>4</sup>)-OH, and  
(10) -C(R<sup>3</sup>)(R<sup>4</sup>)-O-C<sub>1-4</sub>alkyl;
- (e) benzoheteroaryl which includes the benzo fused analogs of  
(d);  
R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>5'</sup> are each independently selected from  
25 the group consisting of  
(a) hydrogen,  
(b) C<sub>1-6</sub>alkyl.

Additional selective cyclooxygenase-2 inhibitors within the  
30 scope of claimed method include:

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as well as compounds disclosed in WO 94/13635, published June 23, 1994; US 5,344,911, issued September 6, 1994; and WO 94/15932, published July 21 1994, all of which are hereby incorporated by  
 5 reference. Additional selective cyclooxygenase-2 inhibitors within the scope of claimed method include Diclofenac, those disclosed in USSN 08/330, filed October 27, 1994; USSN 08/361,268, filed December 21, 1994 and USSN 08/443,620, filed May 18, 1995, all of which are incorporated by method.

10

For purposes of this specification alkyl is defined to include linear, branched, and cyclic structures, with C<sub>1</sub>-6alkyl including methyl, ethyl, propyl, 2-propyl, s- and t-butyl, butyl, pentyl, hexyl, 1,1-dimethylethyl, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

15 Similarly, C<sub>1</sub>-6alkoxy is intended to include alkoxy groups of from 1 to 6 carbon atoms of a straight, branched, or cyclic configuration.

Examples of lower alkoxy groups include methoxy, ethoxy, propoxy, isopropoxy, cyclopropyloxy, cyclohexyloxy, and the like. Likewise, C<sub>1</sub>-6alkylthio is intended to include alkylthio groups of from 1 to 6

20 carbon atoms of a straight, branched or cyclic configuration. Examples of lower alkylthio groups include methylthio, propylthio, isopropylthio, cycloheptylthio, etc. By way of illustration, the propylthio group signifies -SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>.

Heteroaryl includes furan, thiophene, pyrrole, isoxazole, isothiazole, pyrazole, oxazole, thiazole, imidazole, 1,2,3-oxadiazole, 1,2,3-thiadiazole, 1,2,3-triazole, 1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,3,4-triazole, 1,2,5-oxadiazole, 1,2,5-thiadiazole, pyridine, pyridazine, pyrimidine, pyrazine, 1,2,4-triazine, 1,3,5-triazine, 1,2,4,5-tetrazine, and the like.



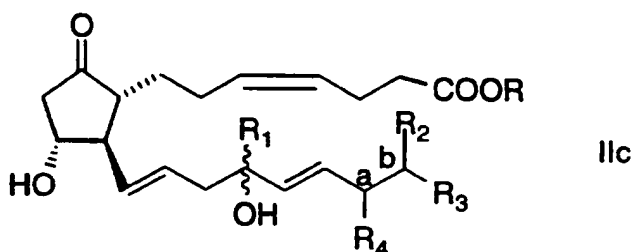
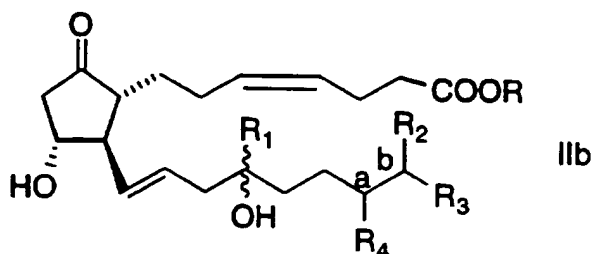
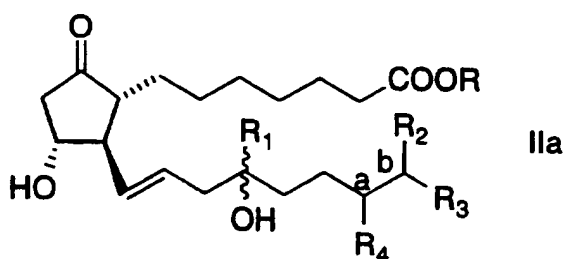
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Exemplifying the invention are:

- (a) 2-(4-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2-cyclopenten-1-one
- 5 (b) 3-(4-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- (c) 3-(4-Fluorophenyl)-4-(4-(aminosulfonyl)phenyl)-2-(5*H*)-furanone,
- (d) 5,5-Dimethyl-3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- 10 (e) 3-(3-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- (f) 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- (g) 5,5-Dimethyl-3-(3-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- 15 (h) 3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- (i) 3-(3,4-Dichlorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- 20 (j) 5,5-Dimethyl-3-(3,4-difluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- (k) 5,5-Dimethyl-3-(3,4-dichlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- (l) 5,5-Dimethyl-3-(4-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- 25 (m) 5,5-Dimethyl-3-(2-naphthyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,
- (n) 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone.
- 30

For purposes of this specification the prostaglandin suitable for the disclosed method includes the compounds of Formula II

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wherein

R is hydrogen or C<sub>1-6</sub>alkyl;

5 R<sub>1</sub> is hydrogen, vinyl or C<sub>1-4</sub>alkyl and the wavy line represents R or S stereochemistry;

10 R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are hydrogen or C<sub>1-4</sub>alkyl or R<sub>2</sub> and R<sub>3</sub> together with carbon b form a cycloalkenyl having 4 to 6 carbon atoms or R<sub>3</sub> and R<sub>4</sub> together with carbons a and b form a cycloalkenyl having 4 to 6 carbons and wherein the a-b bond can be saturated or unsaturated.

These useful prostaglandins include misoprostol,  $\pm$ methyl 11 $\alpha$ , 16-dihydroxy-16-methyl-9-oxoprost 13E-en-1-oate; enisoprost and methyl-7-[2B-[6-(1-cyclopenten-1-yl)-4-hydroxy-4-methyl-1E, 5E-hexadienyl]-3 $\alpha$ -hydroxy-5-oxo 1R, 1 $\alpha$ -cyclopentyl]-4Z-heptenoate.

15 Prostaglandins within the scope of the invention also include arbaprostil,

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enprostil, rioprostol, nocloprost, mexiprostil, ornoprostol, dimoxaprost, tiprostanide, and rosaprostol.

In one aspect applicants method includes the use of the prostaglandin misoprostol with Diclofenac.

5           With regard to the illustrated structures of formula II, the dashed line indicates the grouping being behind the plane of the paper and the solid, blackened triangular shape indicates that the group is in front of the plane of the paper.

10           The prostaglandins useful in the composition of the invention herein can be prepared by known reaction schemes such as by the methods taught in U.S. Pat. Nos. 3,965,143; 4,271,314; and 4,683,328 and in an article by P.W. Collins and J.W. Djurie, Chem. Rev. 1993, 93, 1533-1564.. The individual isomers can be obtained by chromatographic separation. The prostaglandin is preferably an orally  
15 available prostaglandin.

          When the prostaglandin is misoprostol, ( $\pm$ ) methyl 11alpha, 16 dihydroxy 16 methyl-9-oxoprost13E-en-1-oate, the misoprostol is present in an amount of about 100 to 200 mcg (micrograms).

20           For purposes of this specification, the anti-ulcer agent shall be defined to include cimetidine, famotidine, omeprazole, ranitidine and the like.

          As appreciated by those of skill in the art, the co-administration of a selective cyclooxygenase-2 inhibitor with an additional active agent, such as a prostaglandin or anti-ulcer agent,  
25 includes situations wherein both active agents are provided in a single dosage form as well as situations wherein the active agents are provided in separate dosage forms. For example, while for patient compliance it may be advantageous to provide both agents in a single dosage form, depending on the particular species selected, it may be advantage to  
30 administer one of the agents three times a day and the other twice a day.

          Some of the compounds described herein contain one or more asymmetric centers and may thus give rise to diastereomers and optical isomers. The present invention is meant to comprehend such possible diastereomers as well as their racemic and resolved,

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enantiomerically pure forms and pharmaceutically acceptable salts thereof.

Some of the compounds described herein contain olefinic double bonds, and unless specified otherwise, are meant to include both  
5 E and Z geometric isomers.

The pharmaceutical compositions of the present invention comprise a compound of Formula I as an active ingredient or a pharmaceutically acceptable salt, thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic  
10 ingredients. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium,  
15 zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion  
20 exchange resins, such as arginine, betaine, caffeine, choline, N,N\_-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine,  
25 piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

It will be understood that in the discussion of methods of treatment which follows, references to the compounds of Formula I are  
30 meant to also include the pharmaceutically acceptable salts.

The Compound of Formula I is useful for the relief of pain, fever and inflammation of a variety of conditions including rheumatic fever, symptoms associated with influenza or other viral infections, common cold, low back and neck pain, dysmenorrhea, headache,

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toothache, sprains and strains, myositis, neuralgia, synovitis, arthritis, including rheumatoid arthritis degenerative joint diseases (osteoarthritis), gout and ankylosing spondylitis, bursitis, burns, injuries, following surgical and dental procedures. In addition, such a  
5 compound may inhibit cellular neoplastic transformations and metastatic tumor growth and hence can be used in the treatment of cancer.

Compounds of formula I may also be useful for the treatment of dementia including pre-senile and senile dementia, and in particular, dementia associated with Alzheimer Disease (ie Alzheimer's dementia).

10 Compounds of formula I will also inhibit prostanoid-induced smooth muscle contraction by preventing the synthesis of contractile prostanoids and hence may be of use in the treatment of dysmenorrhea, premature labor and asthma.

By virtue of its high cyclooxygenase-2 (COX-2) activity  
15 and/or its selectivity for cyclooxygenase-2 over cyclooxygenase-1 (COX-1) as defined above, compounds of formula I will prove useful as an alternative to conventional non-steroidal antiinflammatory drugs (NSAID'S) particularly where such non-steroidal antiinflammatory drugs may be contra-indicated such as in patients with peptic ulcers,  
20 gastritis, regional enteritis, ulcerative colitis, diverticulitis or with a recurrent history of gastrointestinal lesions; GI bleeding, coagulation disorders including anemia such as hypoprothrombinemia, haemophilia or other bleeding problems (including those relating to reduced or impaired platelet function); kidney disease (eg impaired renal function);  
25 those prior to surgery or taking anticoagulants; and those susceptible to NSAID induced asthma.

Similarly, compounds of formula I, will be useful as a partial or complete substitute for conventional NSAID'S in preparations wherein they are presently co-administered with other agents or  
30 ingredients. Thus in further aspects, the invention encompasses pharmaceutical compositions for treating cyclooxygenase-2 mediated diseases as defined above comprising a non-toxic therapeutically effective amount of the compound of Formula I as defined above and one or more ingredients such as another pain reliever including

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acetaminophen or phenacetin; a potentiator including caffeine; an H<sub>2</sub>-antagonist, aluminum or magnesium hydroxide, simethicone, a decongestant including phenylephrine, phenylpropanolamine, pseudophedrine, oxymetazoline, ephinephrine, naphazoline, xylometazoline, propylhexedrine, or levo-desoxyephedrine; an antiitussive including codeine, hydrocodone, caramiphen, carbetapentane, or dextramethorphan; a diuretic; a sedating or non-sedating antihistamine. In addition the invention encompasses a method of treating cyclooxygenase mediated diseases comprising:

5 administration to a patient in need of such treatment a non-toxic therapeutically effect amount of the compound of Formula I, optionally co-administered with one or more of such ingredients as listed immediately above.

10

As indicated above, pharmaceutical compositions for treating cyclooxygenase-2 mediated diseases as defined may include one or more ingredients as listed above as well as a compound of formula II.

15

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by

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known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also  
5 be coated by the technique described in the U.S. Patent 4,256,108; 4,166,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert  
10 solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredients is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in  
15 admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethyl-cellulose, methylcellulose, hydroxy-propylmethycellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a  
20 naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial  
25 esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-  
30 propyl, p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil,

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sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral  
5 preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending  
10 agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also  
15 be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol  
20 anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening  
25 agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using  
30 those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed



- 15 -

are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of formula I may also be administered in the form of a suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of Formula I are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

Specific cyclooxygenase-2 inhibitor dosage levels of the order of from about 0.01 mg to about 140 mg/kg of body weight per day are useful in the treatment of the above-indicated conditions, or alternatively about 0.5 mg to about 7 g per patient per day. For example, inflammation may be effectively treated by the administration of from about 0.01 to 50 mg of the compound per kilogram of body weight per day, or alternatively about 0.5 mg to about 3.5 g per patient per day.

With regard to the prostaglandins, typical dosages may be as much as 25 to 1600 µg per day; more typically 200 to 800 µg per day (eg 200, 400, 600 or 800 µg per day. Single dosage forms may typically contain 5, 25, 50, 100, 200, 250, 400 or 500 µg per tablet.

When the prostaglandin is misoprostol, (±) methyl 11α, 16 dihydroxy 16 methyl-9-oxoprost13E-en-1-oate, the misoprostol is present in the dosage form (eg tablet) in an amount of about 100 to 200 µg. See, for example, a Physicians Desk Reference (PDR).

With regard to anti-ulcer agents, such as those described above, the dosages may typically range from 10 to 800 mg per day or more, with single dosages containing 10, 20, 30, 100, 200, 400 or 800

- 16 -

mg of active agent. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral  
5 administration of humans may contain from 0.5 mg to 5 g of specific cyclooxygenase-2 inhibitor compounded with a prostaglandin of formula II and an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from  
10 about 1 mg to about 500 mg of an active ingredient, typically 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 800 mg, or 1000 mg of specific cyclooxygenase-2 inhibitor.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors  
15 including the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

#### 20 Assays for Determining Biological Activity

The compound of Formula I can be tested using the following assays to determine their cyclooxygenase-2 inhibiting activity.

#### 25 INHIBITION OF CYCLOOXYGENASE ACTIVITY

Compounds are tested as inhibitors of cyclooxygenase activity in whole cell cyclooxygenase assays. Both of these assays measure prostaglandin E<sub>2</sub> synthesis in response to arachidonic acid, using a radioimmunoassay. Cells used for these assays are human  
30 osteosarcoma 143 cells (which specifically express COX-2) and human U-937 cells (which specifically express COX-1). In these assays, 100% activity is defined as the difference between prostaglandin E<sub>2</sub> synthesis in the absence and presence of arachidonate.

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### Whole Cell Assays

- For cyclooxygenase assays, osteosarcoma cells are cultured in 1 mL of media in 24-well multidishes (Nunc) until confluent ( $1-2 \times 10^5$  cells/well). U-937 cells are grown in spinner flasks and
- 5 resuspended to a final density of  $1.5 \times 10^6$  cells/mL in 24-well multidishes (Nunc). Following washing and resuspension of osteosarcoma and U-937 cells in 1 mL of HBSS, 1  $\mu$ L of a DMSO solution of test compound or DMSO vehicle is added, and samples gently mixed. All assays are performed in triplicate. Samples are then
- 10 incubated for 5 or 15 minutes at 37C, prior to the addition of arachidonic acid. Arachidonic acid (peroxide-free, Cayman Chemical) is prepared as a 10 mM stock solution in ethanol and further diluted 10-fold in HBSS. An aliquot of 10  $\mu$ L of this diluted solution is added to the cells to give a final arachidonic acid concentration of 10  $\mu$ M.
- 15 Control samples are incubated with ethanol vehicle instead of arachidonic acid. Samples are again gently mixed and incubated for a further 10 min. at 37C. For osteosarcoma cells, reactions are then stopped by the addition of 100  $\mu$ L of 1N HCl, with mixing and by the rapid removal of the solution from cell monolayers. For U-937 cells,
- 20 reactions are stopped by the addition of 100  $\mu$ L of 1N HCl, with mixing. Samples are then neutralized by the addition of 100  $\mu$ L of 1N NaOH and PGE<sub>2</sub> levels measured by radioimmunoassay.

### Whole cell assays for COX-2 and COX-1 using CHO transfected cell

25 lines

- Chinese hamster ovary (CHO) cell lines which have been stably transfected with an eukaryotic expression vector pCDNAIII containing either the human COX-1 or COX-2 cDNA's are used for the assay.
- 30 These cell lines are referred to as CHO [hCOX-1] and CHO [hCOX-2], respectively. For cyclooxygenase assays, CHO[hCOX-1] cells from suspension cultures and CHO[hCOX-2] cells prepared by trypsinization of adherent cultures are harvested by centrifugation (300 x g, 10 min) and washed once in HBSS containing 15 mM HEPES, pH 7.4, and

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resuspended in HBSS, 15 mM HEPES, pH 7.4, at a cell concentration of  $1.5 \times 10^6$  cells/ml. Drugs to be tested are dissolved in DMSO to 66.7-fold the highest test drug concentration. Compounds are typically tested at 8 concentrations in duplicate using serial 3-fold serial dilutions in

5 DMSO of the highest drug concentration. Cells ( $0.3 \times 10^6$  cells in 200  $\mu$ l) are preincubated with 3  $\mu$ l of the test drug or DMSO vehicle for 15 min at 37C. Working solutions of peroxide-free AA (5.5  $\mu$ M and 110  $\mu$ M AA for the CHO [hCOX-1] and CHO [COX-2] assays, respectively) are prepared by a 10-fold dilution of a concentrated AA solution in

10 ethanol into HBSS containing 15 mM HEPES, pH 7.4. Cells are then challenged in the presence or absence of drug with the AA/HBSS solution to yield a final concentration of 0.5  $\mu$ M AA in the CHO[hCOX-1] assay and a final concentration of 10  $\mu$ M AA in the CHO[hCOX-2] assay. The reaction is terminated by the addition of 10  $\mu$ l 1 N HCl

15 followed by neutralization with 20  $\mu$ l of 0.5 N NaOH. The samples are centrifuged at 300 x g at 4C for 10 min, and an aliquot of the clarified supernatant is appropriately diluted for the determination of PGE<sub>2</sub> levels using an enzyme-linked immunoassay for PGE<sub>2</sub> (Correlate PGE<sub>2</sub> enzyme immunoassay kit, Assay Designs, Inc.). Cyclooxygenase activity

20 in the absence of test compounds is determined as the difference in PGE<sub>2</sub> levels of cells challenged with arachidonic acid versus the PGE<sub>2</sub> levels in cells mock-challenged with ethanol vehicle. Inhibition of PGE<sub>2</sub> synthesis by test compounds is calculated as a percentage of the activity in the presence of drug versus the activity in the positive control

25 samples.

#### Assay of COX-1 Activity from U937 cell microsomes

U 937 cells are pelleted by centrifugation at 500 x g for 5 min and

30 washed once with phosphate-buffered saline and repelleted. Cells are resuspended in homogenization buffer consisting of 0.1 M Tris-HCl, pH 7.4, 10 mM EDTA, 2  $\mu$ g/ml leupeptin, 2  $\mu$ g/ml soybean trypsin inhibitor, 2  $\mu$ g/ml aprotinin and 1 mM phenyl methyl sulfonyl fluoride. The cell suspension is sonicated 4 times for 10 sec and is centrifuged at

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10,000 x g for 10 min at 4° C. The supernatant is centrifuged at 100,000 x g for 1 hr at 4 ° C. The 100,000 x g microsomal pellet is resuspended in 0.1 M Tris-HCl, pH 7.4, 10 mM EDTA to approximately 7 mg protein/ml and stored at -80° C.

5

Microsomal preparations are thawed immediately prior to use, subjected to a brief sonication, and then diluted to a protein concentration of 125 µg/ml in 0.1 M Tris-HCl buffer, pH 7.4 containing 10 mM EDTA, 0.5 mM phenol, 1 mM reduced glutathione and 1 µM hematin. Assays are performed in duplicate in a final volume of 250 µl. Initially, 5 µl of DMSO vehicle or drug in DMSO are added to 20 µl of 0.1 M Tris-HCl buffer, pH 7.4 containing 10 mM EDTA in wells of a 96-deepwell polypropylene titre plate. 200 µl of the microsomal preparation are then added and pre-incubated for 15 min at room temperature before addition of 25 µl of 1 M arachidonic acid in 0.1 M Tris-HCl and 10 mM EDTA, pH 7.4. Samples are incubated for 40 min at room temperature and the reaction is stopped by the addition of 25 µl of 1 N HCl. Samples are neutralized with 25 µl of 1 N NaOH prior to quantitation of PGE<sub>2</sub> content by radioimmunoassay (Dupont-NEN or Amersham assay kits). Cyclooxygenase activity is defined as the difference between PGE<sub>2</sub> levels in samples incubated in the presence of arachidonic acid and ethanol vehicle.

#### Assay of the activity of purified human COX-2

25

The enzyme activity is measured using a chromogenic assay based on the oxidation of N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) during the reduction of PGG<sub>2</sub> to PGH<sub>2</sub> by COX-2 (Copeland et al. (1994) Proc. Natl. Acad. Sci. 91, 11202-11206).

30

Recombinant human COX-2 is purified from Sf9 cells as previously described (Percival et al (1994) Arch. Biochem. Biophys. 15, 111-118). The assay mixture (180 µL) contains 100 mM sodium phosphate, pH 6.5, 2 mM genapol X-100, 1 µM hematin, 1 mg/ml gelatin, 80-100

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units of purified enzyme (One unit of enzyme is defined as the amount of enzyme required to produce an O.D. change of 0.001/min at 610 nm) and 4  $\mu$ L of the test compound in DMSO. The mixture is pre-incubated at room temperature (22°C) for 15 minutes prior to initiation of the enzymatic reaction by the addition of 20  $\mu$ L of a sonicated solution of 1 mM arachidonic acid (AA) and 1 mM TMPD in assay buffer (without enzyme or hematin). The enzymatic activity is measured by estimation of the initial velocity of TMPD oxidation over the first 36 sec of the reaction. A non-specific rate of oxidation is observed in the absence of enzyme (0.007 - 0.010 O.D. /min) and is subtracted before the calculation of the % inhibition. IC<sub>50</sub> values are derived from 4-parameter least squares non-linear regression analysis of the log-dose vs % inhibition plot.

15

## HUMAN WHOLE BLOOD ASSAY

### Rationale

Human whole blood provides a protein and cell-rich milieu appropriate for the study of biochemical efficacy of anti-inflammatory compounds such as selective COX-2 inhibitors. Studies have shown that normal human blood does not contain the COX-2 enzyme. This is consistent with the observation that COX-2 inhibitors have no effect on PGE<sub>2</sub> production in normal blood. These inhibitors are active only after incubation of human whole blood with LPS, which induces COX-2. This assay can be used to evaluate the inhibitory effect of selective COX-2 inhibitors on PGE<sub>2</sub> production. As well, platelets in whole blood contain a large amount of the COX-1 enzyme. Immediately following blood clotting, platelets are activated through a thrombin-mediated mechanism. This reaction results in the production of thromboxane B<sub>2</sub> (TxB<sub>2</sub>) via activation of COX-1. Thus, the effect of test compounds on TxB<sub>2</sub> levels following blood clotting can be examined and used as an index for COX-1 activity. Therefore, the degree of selectivity by the test compound can be determined by

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measuring the levels of PGE<sub>2</sub> after LPS induction (COX-2) and TxB<sub>2</sub> following blood clotting (COX-1) in the same assay.

### Method

#### 5 A. COX-2 (LPS-induced PGE<sub>2</sub> production)

Fresh blood is collected in heparinized tubes by venipuncture from both male and female volunteers. The subjects have no apparent inflammatory conditions and have not taken any NSAIDs for at least 7 days prior to blood collection. Plasma is immediately obtained from a  
10 2mL blood aliquot to use as blank (basal levels of PGE<sub>2</sub>). The remaining blood is incubated with LPS (100 µg/ml final concentration, Sigma Chem, #L-2630 from E. coli; diluted in 0.1% BSA (Phosphate buffered saline) for 5 minutes at room temperature. Five hundred µL aliquots of blood are incubated with either 2µL of vehicle (DMSO) or  
15 2µL of a test compound at final concentrations varying from 10nM to 30µM for 24 hours at 37°C. At the end of the incubation, the blood is centrifuged at 12,000 x g for 5 minutes to obtain plasma. A 100µL aliquot of plasma is mixed with 400µL of methanol for protein precipitation. The supernatant is obtained and is assayed for PGE<sub>2</sub>  
20 using a radioimmunoassay kit (Amersham, RPA#530) after conversion of PGE<sub>2</sub> to its methyl oximate derivative according to the manufacturer's procedure.

#### 25 B. COX-1 (Clotting-induced TxB<sub>2</sub> production)

Fresh blood is collected into vacutainers containing no anticoagulants. Aliquots of 500µL are immediately transferred to siliconized microcentrifuge tubes preloaded with 2µL of either DMSO or a test compound at final concentrations varying from 10nM to 30µM. The tubes are vortexed and incubated at 37°C for 1 hour to allow blood  
30 to clot. At the end of incubation, serum is obtained by centrifugation (12,000 x g for 5 min.). A 100µL aliquot of serum is mixed with 400µL of methanol for protein precipitation. The supernatant is obtained and is assayed for TxB<sub>2</sub> using a enzyme immunoassay kit (Cayman, #519031) according to the manufacturer's instruction.

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## RAT PAW EDEMA ASSAY

### Protocol

- 5        Male Sprague-Dawley rats (150-200 g) are fasted overnight and are given, po, either vehicle (1% methocel or 5% Tween 80) or a test compound. One hr later, a line is drawn using a permanent marker at the level above the ankle in one hind paw to define the area of the paw to be monitored. The paw volume ( $V_0$ ) is measured using a
- 10    plethysmometer (Ugo-Basile, Italy) based on the principle of water displacement. The animals are then injected subplantarily with 50  $\mu$ l of 1% carrageenan solution in saline (FMC Corp, Maine) into the paw using an insulin syringe with a 25-gauge needle (i.e. 500  $\mu$ g carrageenan per paw). Three hr later, the paw volume ( $V_3$ ) is
- 15    measured and the increases in paw volume ( $V_3 - V_0$ ) are calculated. The animals are sacrificed by CO<sub>2</sub> asphyxiation and the absence or presence of stomach lesions scored. Data is compared with the vehicle-control values and percent inhibition calculated. All treatment groups are coded to eliminate observer bias.
- 20

## NSAID-INDUCED GASTROPATHY IN RATS

### Rationale

- 25        The major side effect of conventional NSAIDs is their ability to produce gastric lesions in man. This action is believed to be caused by inhibition of Cox-1 in the gastrointestinal tract. Rats are particularly sensitive to the actions of NSAIDs. In fact, rat models have been used commonly in the past to evaluate the gastrointestinal side effects of
- 30    current conventional NSAIDs. In the present assay, NSAID-induced gastrointestinal damage is observed by measuring fecal <sup>51</sup>Cr excretion after systemic injection of <sup>51</sup>Cr-labeled red blood cells. Fecal <sup>51</sup>Cr excretion is a well-established and sensitive technique to detect gastrointestinal integrity in animals and man.



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### Methods

Male Sprague Dawley rats (150 - 200 g) are administered orally a test compound either once (acute dosing) or b.i.d. for 5 days (chronic dosing). Immediately after the administration of the last dose, the rats are injected via a tail vein with 0.5 mL of  $^{51}\text{Cr}$ -labeled red blood cells from a donor rat. The animals are placed individually in metabolism cages with food and water *ad lib*. Feces are collected for a 48 h period and  $^{51}\text{Cr}$  fecal excretion is calculated as a percent of total injected dose.

$^{51}\text{Cr}$ -labeled red blood cells are prepared using the following procedures. Ten mL of blood is collected in heparinized tubes via the vena cava from a donor rat. Plasma is removed by centrifugation and replenished with equal volume of HBSS. The red blood cells are incubated with 400 Ci of sodium  $^{51}\text{Cr}$  chromate for 30 min. at 37C. At the end of the incubation, the red blood cells are washed twice with 20 mL HBSS to remove free sodium  $^{51}\text{Cr}$  chromate. The red blood cells are finally reconstituted in 10 mL HBSS and 0.5 mL of the solution (about 20 Ci) is injected per rat.

## 20 PROTEIN-LOSING GASTROPATHY IN SQUIRREL MONKEYS

### Rationale

Protein-losing gastropathy (manifested as appearance of circulating cells and plasma proteins in the GI tract) is a significant and dose-limiting adverse response to standard non-steroidal anti-inflammatory drugs (NSAIDs). This can be quantitatively assessed by intravenous administration of  $^{51}\text{CrCl}_3$  solution. This isotopic ion can avidly bind to cell and serum globins and cell endoplasmic reticulum. Measurement of radioactivity appearing in feces collected for 24 h after administration of the isotope thus provides a sensitive and quantitative index of protein-losing gastropathy.

### Methods

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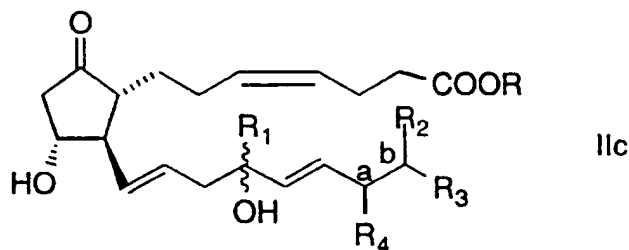
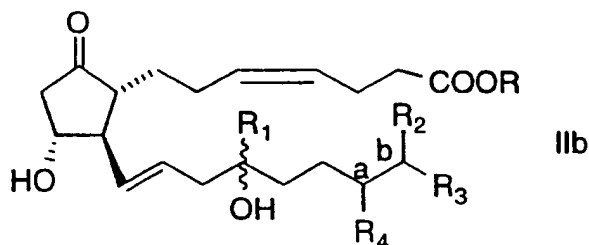
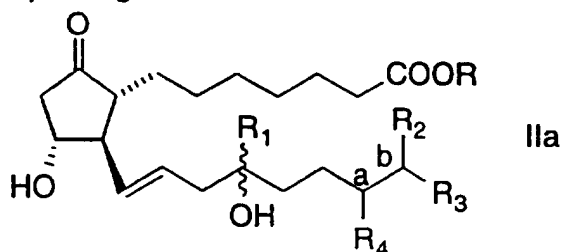
Groups of male squirrel monkeys (0.8 to 1.4 kg) are treated by gavage with either 1% methocell or 5% Tween 80 in H<sub>2</sub>O vehicles, (3mL/kg b.i.d.) or test compounds at doses from 1 - 100 mg/kg b.i.d. for 5 days. Intravenous <sup>51</sup>Cr (5Ci/kg in 1 ml/kg phosphate buffer saline (PBS)) is administered 1 h after the last drug/vehicle dose, and feces collected for 24 h in a metabolism cage and assessed for excreted <sup>51</sup>Cr by gamma-counting. Venous blood is sampled 1 h and 8 h after the last drug dose, and plasma concentrations of drug measured by RP-HPLC.

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WHAT IS CLAIMED IS:

1. A pharmaceutical composition for treating cyclo-oxygenase-2 mediated disease comprising an agent selected from an anti-ulcer agent and a prostaglandin of formula II



wherein

R is hydrogen or C<sub>1</sub>-6alkyl;

- 10 R<sub>1</sub> is hydrogen, vinyl or C<sub>1</sub>-4alkyl and the wavy line represents R or S stereochemistry;

R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are hydrogen or C<sub>1</sub>-4alkyl or R<sub>2</sub> and R<sub>3</sub> together with carbon b form a cycloalkenyl having 4 to 6 carbon atoms or R<sub>3</sub> and R<sub>4</sub> together with carbons a and b form a cycloalkenyl having 4 to 6 carbons

- 15 and wherein the a-b bond can be saturated or unsaturated;

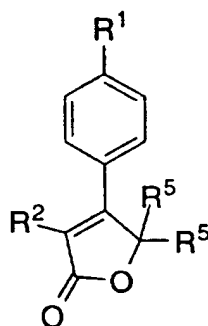
together with a selective cyclooxygenase-2 inhibitor.

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2. A composition of Claim 1 wherein the prostaglandin is selected from misoprostol, and methyl-7-[2B-[6-(1-cyclopenten-1-yl)-4-hydroxy-4-methyl-1E, 5E-hexadienyl]-3 $\alpha$ -hydroxy-5-oxo 1R, 1 $\alpha$ -cyclopentyl]-4Z-heptenoate.

5

3. A composition according to Claim 1 or 2 wherein the selective cyclooxygenase-2 inhibitor is a compound of Formula Ia



Ia

10

or pharmaceutically acceptable salts thereof wherein:

R<sup>1</sup> is selected from the group consisting of

- (a) S(O)<sub>2</sub>CH<sub>3</sub>,
- (b) S(O)<sub>2</sub>NH<sub>2</sub>,
- (c) S(O)<sub>2</sub>NHC(O)CF<sub>3</sub>,

15

R<sup>2</sup> is selected from the group consisting of

- (a) C<sub>1-6</sub>alkyl,
- (b) C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, and C<sub>7</sub>, cycloalkyl,
- (c) mono-, di- or tri-substituted phenyl or naphthyl wherein the substituent is selected from the group consisting of selected from the group consisting of
  - (1) hydrogen,
  - (2) halo,
  - (3) C<sub>1-6</sub>alkoxy,
  - (4) C<sub>1-6</sub>alkylthio,
  - (5) CN,

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- 5 (6) CF<sub>3</sub>,  
(7) C<sub>1</sub>-6alkyl,  
(8) N<sub>3</sub>,  
(9) -CO<sub>2</sub>H,  
(10) -CO<sub>2</sub>-C<sub>1</sub>-4alkyl,  
(11) -C(R<sup>3</sup>)(R<sup>4</sup>)-OH,  
(12) -C(R<sup>3</sup>)(R<sup>4</sup>)-O-C<sub>1</sub>-4alkyl, and  
(13) -C<sub>1</sub>-6alkyl-CO<sub>2</sub>-R<sup>3</sup>;  
10 (d) heteroaryl  
(e) benzoheteroaryl  
R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>5'</sup> are each independently selected from  
the group consisting of  
(a) hydrogen,  
(b) C<sub>1</sub>-6alkyl.

15

4. A composition according to Claim 3 wherein  
R<sup>1</sup> is selected from the group consisting of  
(a) S(O)<sub>2</sub>CH<sub>3</sub>, and  
(b) S(O)<sub>2</sub>NH<sub>2</sub>,  
20 R<sup>2</sup> is  
mono or di-substituted phenyl wherein the substituents are  
selected from the group consisting of  
(1) hydrogen,  
(2) halo, selected from the group consisting of fluoro,  
25 chloro and bromo; and  
R<sup>5</sup> and R<sup>5'</sup> are each hydrogen.

5. A composition according to Claim 1 or 2 wherein the  
selective cyclooxygenase-2 inhibitor is  
30 (a) 2-(4-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2-  
cyclopenten-1-one  
(b) 3-(4-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-  
(5*H*)-furanone,

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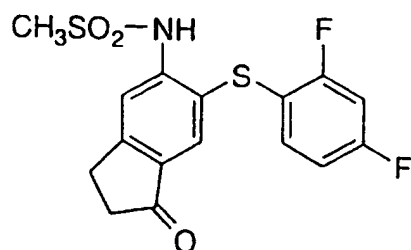
- (c) 3-(4-Fluorophenyl)-4-(4-(aminosulfonyl)phenyl)-2-(5*H*)-furanone,  
(d) 5,5-Dimethyl-3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
5 (e) 3-(3-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(f) 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(g) 5,5-Dimethyl-3-(3-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
10 (h) 3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(i) 3-(3,4-Dichlorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
15 (j) 5,5-Dimethyl-3-(3,4-difluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(k) 5,5-Dimethyl-3-(3,4-dichlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(l) 5,5-Dimethyl-3-(4-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
20 (m) 5,5-Dimethyl-3-(2-naphthyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(n) 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone.

25

6. A composition according to Claim 1 or 2 wherein the selective cyclooxygenase-2 inhibitor is

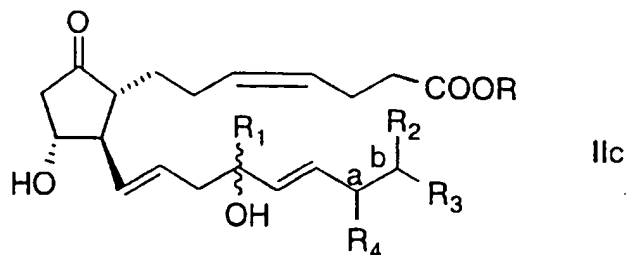
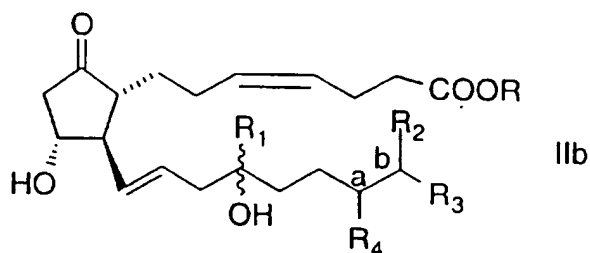
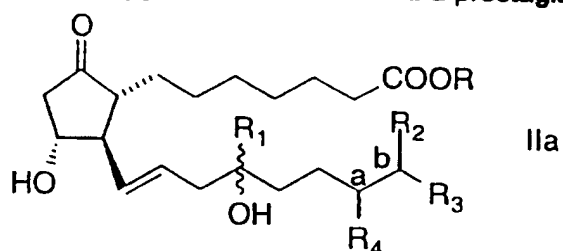
- 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
30 3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,

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or a pharmaceutically acceptable salt thereof.

7. A system for treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers or protecting the gastric mucosa comprising a selective cyclooxygenase-2 inhibitor and a prostaglandin of formula II



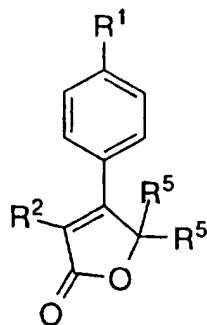
- wherein
- 10 R is hydrogen or C<sub>1</sub>-6alkyl;

- 30 -

R<sub>1</sub> is hydrogen, vinyl or C<sub>1</sub>-4alkyl and the wavy line represents R or S stereochemistry;

- R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are hydrogen or C<sub>1</sub>-4alkyl or R<sub>2</sub> and R<sub>3</sub> together with carbon b form a cycloalkenyl having 4 to 6 carbon atoms or R<sub>3</sub> and R<sub>4</sub> together with carbons a and b form a cycloalkenyl having 4 to 6 carbons and wherein the a-b bond can be saturated or unsaturated, in a form for co-administration with said selective cyclooxygenase-2 inhibitor.

8. A system according to Claim 7 wherein the selective cyclooxygenase-2 inhibitor is a compound of Formula Ia



Ia

or pharmaceutically acceptable salts thereof wherein:

- R<sup>1</sup> is selected from the group consisting of
- S(O)<sub>2</sub>CH<sub>3</sub>,
  - S(O)<sub>2</sub>NH<sub>2</sub>,
  - S(O)<sub>2</sub>NHC(O)CF<sub>3</sub>,
- R<sup>2</sup> is selected from the group consisting of
- C<sub>1</sub>-6alkyl,
  - C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, and C<sub>7</sub>, cycloalkyl,
  - mono-, di- or tri-substituted phenyl or naphthyl wherein the substituent is selected from the group consisting of
- selected from the group consisting of
- hydrogen,
  - halo,
  - C<sub>1</sub>-6alkoxy,



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- 5 (4) C<sub>1</sub>-6alkylthio,  
 (5) CN,  
 (6) CF<sub>3</sub>,  
 (7) C<sub>1</sub>-6alkyl,  
 (8) N<sub>3</sub>,  
 (9) -CO<sub>2</sub>H,  
 (10) -CO<sub>2</sub>-C<sub>1</sub>-4alkyl,  
 (11) -C(R<sup>3</sup>)(R<sup>4</sup>)-OH,  
 (12) -C(R<sup>3</sup>)(R<sup>4</sup>)-O-C<sub>1</sub>-4alkyl, and  
 10 (13) -C<sub>1</sub>-6alkyl-CO<sub>2</sub>-R<sup>3</sup>;  
 (d) heteroaryl,  
 (e) benzoheteroaryl,  
 R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>5'</sup> are each independently selected from  
 the group consisting of  
 15 (a) hydrogen,  
 (b) C<sub>1</sub>-6alkyl.

9. A system according to Claim 8 wherein  
 R<sup>1</sup> is selected from the group consisting of  
 20 (a) S(O)<sub>2</sub>CH<sub>3</sub>, and  
 (b) S(O)<sub>2</sub>NH<sub>2</sub>,  
 R<sup>2</sup> is  
 mono or di-substituted phenyl wherein the substituents are  
 selected from the group consisting of  
 25 (1) hydrogen,  
 (2) halo, selected from the group consisting of fluoro,  
 chloro and bromo; and  
 R<sup>5</sup> and R<sup>5'</sup> are each hydrogen.

- 30 10. A system according to Claim 7 wherein the selective  
 cyclooxygenase-2 inhibitor is  
 (a) 2-(4-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2-  
 cyclopenten-1-one

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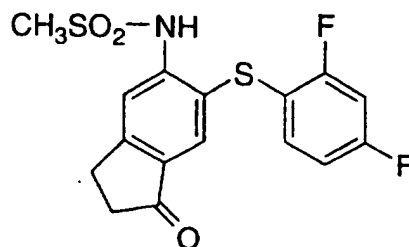
- (b) 3-(4-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(c) 3-(4-Fluorophenyl)-4-(4-(aminosulfonyl)phenyl)-2-(5*H*)-furanone,  
5 (d) 5,5-Dimethyl-3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(e) 3-(3-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(f) 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
10 (g) 5,5-Dimethyl-3-(3-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(h) 3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
15 (i) 3-(3,4-Dichlorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(j) 5,5-Dimethyl-3-(3,4-difluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(k) 5,5-Dimethyl-3-(3,4-dichlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
20 (l) 5,5-Dimethyl-3-(4-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(m) 5,5-Dimethyl-3-(2-naphthyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone, or  
25 (n) 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone.

11. A system according to Claim 7 wherein the selective cyclooxygenase-2 inhibitor is

- 30 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
3-(3,4-Difluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,

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3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(5H)-  
furanone, or



or a pharmaceutically acceptable salt thereof.

5

12. A co-administrable combination of a prostaglandin and a selective cyclooxygenase-2 inhibitor, said selective cyclooxygenase-2 inhibitor being at least 200 times more potent against cyclooxygenase-2 than against cyclooxygenase-1 as measured by the ratio of said inhibitor's IC<sub>50</sub> for cyclooxygenase-1 divided by said inhibitor's IC<sub>50</sub> for cyclooxygenase-2, for use in treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers.

15

13. A co-administrable combination of a prostaglandin and a selective cyclooxygenase-2 inhibitor, said selective cyclooxygenase-2 inhibitor being at least 200 times more potent against cyclooxygenase-2 than against cyclooxygenase-1 as measured by the ratio of said inhibitor's IC<sub>50</sub> for cyclooxygenase-1 divided by said inhibitor's IC<sub>50</sub> for cyclooxygenase-2, for use in treating cyclooxygenase-2 mediated disease while promoting the healing of gastric mucosa.

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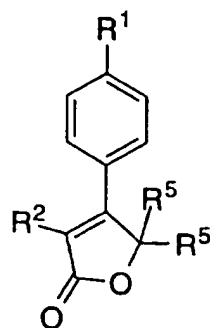
14. Use of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor in the manufacture of a medicament in which the agent and inhibitor are co-administrable, for treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers.

25

15. A use of Claim 14 wherein the anti-ulcer agent is selected from cimetidine, famotidine, omeprazole and ranitidine.

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16. A use according to Claim 15 wherein the selective cyclooxygenase-2 inhibitor is a compound of Formula Ia



Ia

or pharmaceutically acceptable salts thereof wherein:

R¹ is selected from the group consisting of

- 10 (a) S(O)₂CH₃,  
 (b) S(O)₂NH₂,  
 (c) S(O)₂NHC(O)CF₃,

R² is selected from the group consisting of

- 15 (a) C₁-6alkyl,  
 (b) C₃, C₄, C₅, C₆, and C₇, cycloalkyl,  
 (c) mono-, di- or tri-substituted phenyl or naphthyl wherein  
 the substituent is selected from the group consisting of

- selected from the group consisting of
- 20 (1) hydrogen,  
 (2) halo,  
 (3) C₁-6alkoxy,  
 (4) C₁-6alkylthio,  
 (5) CN,  
 (6) CF₃,  
 25 (7) C₁-6alkyl,  
 (8) N₃,  
 (9) -CO₂H,

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- (10)  $-\text{CO}_2\text{-C}_{1-4}\text{alkyl}$ ,  
(11)  $-\text{C}(\text{R}^3)(\text{R}^4)\text{-OH}$ ,  
(12)  $-\text{C}(\text{R}^3)(\text{R}^4)\text{-O-C}_{1-4}\text{alkyl}$ , and  
(13)  $-\text{C}_{1-6}\text{alkyl-CO}_2\text{-R}^3$ ;  
5 (d) heteroaryl  
(e) benzoheteroaryl  
R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>5'</sup> are each independently selected from  
the group consisting of  
(a) hydrogen,  
10 (b) C<sub>1-6</sub>alkyl.

17. A use according to Claim 16 wherein  
R<sup>1</sup> is selected from the group consisting of  
(a)  $\text{S}(\text{O})_2\text{CH}_3$ , and  
15 (b)  $\text{S}(\text{O})_2\text{NH}_2$ ,  
R<sup>2</sup> is  
mono or di-substituted phenyl wherein the substituents are  
selected from the group consisting of  
(1) hydrogen,  
20 (2) halo, selected from the group consisting of fluoro,  
chloro and bromo; and  
R<sup>5</sup> and R<sup>5'</sup> are each hydrogen.

18. A use according to Claim 16 wherein the  
25 selective cyclooxygenase-2 inhibitor is  
(a) 2-(4-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2-  
cyclopenten-1-one  
(b) 3-(4-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-  
(5H)-furanone,  
30 (c) 3-(4-Fluorophenyl)-4-(4-(aminosulfonyl)phenyl)-2-  
(5H)-furanone,  
(d) 5,5-Dimethyl-3-(4-fluorophenyl)-4-(4-  
methylsulfonyl)phenyl)-2-(5H)-furanone,

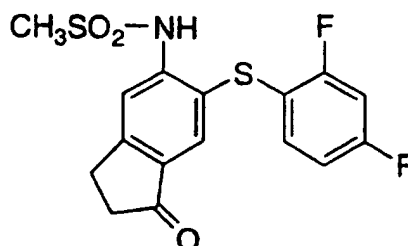
- 36 -

- (e) 3-(3-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(f) 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
5 (g) 5,5-Dimethyl-3-(3-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(h) 3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
10 (i) 3-(3,4-Dichlorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(j) 5,5-Dimethyl-3-(3,4-difluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(k) 5,5-Dimethyl-3-(3,4-dichlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
15 (l) 5,5-Dimethyl-3-(4-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(m) 5,5-Dimethyl-3-(2-naphthyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
(n) 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-  
20 furanone.

19. A use according to Claim 14 wherein the selective cyclooxygenase-2 inhibitor is

- 25 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone,  
3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone, and  
3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone, and

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or a pharmaceutically acceptable salt thereof.

20. Use of a prostaglandin and a selective cyclooxygenase-2 inhibitor, as defined in Claim 1, 2, 3, 4, 5, 6 or 7, as co-administrable agents for treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers or protecting the gastric mucosa.

21. A pharmaceutical composition for treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers comprising an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor.

22. A composition according to Claim 21, wherein the anti-ulcer agent is selected from cimetidine, famotidine, omeprazole and ranitidine.

23. A composition according to Claim 21 or 22, wherein the selective cyclooxygenase-2 inhibitor is a compound of formula Ia or a pharmaceutically acceptable salt thereof, as defined in Claim 16, 17, 18 or 19.

24. A method of treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers comprising the co-administration of a prostaglandin and a selective cyclooxygenase-2 inhibitor, both as defined in Claim 1, 2, 3, 4, 5, 6 or 7.

25. A method of treating cyclooxygenase-2 mediated disease while protecting the gastric mucosa comprising the co-administration of a prostaglandin and a selective cyclooxygenase-2 inhibitor, both as defined in Claim 1, 2, 3, 4, 5, 6 or 7.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA 96/00638

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/557 //(A61K31/557,31:34)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 00501 A (MERCK FROSST CANADA INC ;DUCHARME YVES (CA); GAUTHIER JACQUES YVES) 5 January 1995 see abstract	1-25
A	--- US 5 015 481 A (FRANZ MICHEL ET AL) 14 May 1991 cited in the application see abstract	1-25
A	--- WO 91 16895 A (SEARLE & CO) 14 November 1991 cited in the application see abstract	1-25
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*A\* document member of the same patent family

Date of the actual completion of the international search

4 February 1997

Date of mailing of the international search report

11.02.97

Name and mailing address of the ISA

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Authorized officer

Leherte, C



# INTERNATIONAL SEARCH REPORT

International Application No  
PC 1/CA 96/00638

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 91 16896 A (SEARLE &amp; CO) 14 November 1991  cited in the application  see abstract</p> <p>-----</p>	1-25

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 96/ 00638

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim(s) 24,25  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☒ Claims Nos.: 1-25  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:  
In view of the large number of compounds which are defined by the wording  
of the claims, the search has been performed on the general idea and  
compounds mentioned in the examples of the description.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC I/CA 96/00638

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
WO-A-9500501	05-01-95	US-A-	5474995	12-12-95
		AU-A-	6197096	31-10-96
		AU-A-	6967494	17-01-95
		BG-A-	100247	28-06-96
		BR-A-	9406979	05-03-96
		CA-A-	2163888	05-01-95
		CA-A-	2176973	25-12-94
		CA-A-	2176974	25-12-94
		CN-A-	1125944	03-07-96
		CZ-A-	9503146	15-05-96
		EP-A-	0705254	10-04-96
		EP-A-	0754687	22-01-97
		FI-A-	956119	19-12-95
		NO-A-	955256	23-02-96
		PL-A-	312196	01-04-96
		US-A-	5536752	16-07-96
		US-A-	5550142	27-08-96
		ZA-A-	9404501	13-03-95
		AU-A-	1269495	01-08-95
		CA-A-	2180651	13-07-95
		WO-A-	9518799	13-07-95
		EP-A-	0739340	30-10-96
		FI-A-	962800	06-09-96
		NO-A-	960393	09-07-96
-----				
US-A-5015481	14-05-91	NONE		
-----				
WO-A-9116895	14-11-91	AT-T-	121625	15-05-95
		AU-A-	7876491	27-11-91
		DE-D-	69109286	01-06-95
		DE-T-	69109286	28-09-95
		EP-A-	0527887	24-02-93
		ES-T-	2071312	16-06-95
		IL-A-	98033	31-07-95
-----				
WO-A-9116896	14-11-91	AU-A-	7793791	27-11-91
		IE-B-	62051	14-12-94
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